IS 5887-8-2 (2002): Methods for Detection of Bacteria Responsible for Food Poisoning, Part 8: Horizontal Method for Enumeration of Coagulase-Positive Staphylococci (Staphylococcus Aureus and other species), Section 2 Technique using rabbit plasma fibrinogen agar medium [FAD 15: Food Hygiene, Safety Management and Other Systems]

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IS 5887 (Part 8/Sec 2) : 2002
ISO 6888-2 : 1999
(Reaffirmed - 2012)

भारतीय मानक

खाद्य विषाक्तता के लिए उत्तरदायी जीवाणुओं के पहचान की पद्धतियाँ

भाग 8 स्कंदित सकारात्मक *स्टेफाइलोकोकाई* की गणना के लिए होरीजेन्टल पद्धति (*स्टेफाईलोकोकस ऑरियस* और अन्य प्रजातियाँ)

अनुभाग 2 खरगोश जीवद्रव्य फाइब्रीनोजन अगार माध्यम का उपयोग करने वाली तकनीक

Indian Standard

METHODS FOR DETECTION OF BACTERIA RESPONSIBLE FOR FOOD POISONING

PART 8 HORIZONTAL METHOD FOR ENUMERATION OF COAGULASE-POSITIVE *STAPHYLOCOCCI* (*STAPHYLOCOCCUS AUREUS* AND OTHER SPECIES)

Section 2 Technique Using Rabbit Plasma Fibrinogen Agar Medium

ICS 07.100.30

BUREAU OF INDIAN STANDARDS
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Price Group 4

NATIONAL FOREWORD

This Indian Standard (Part 8/Sec 2) which is identical with ISO 6888-2 : 1999 'Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) — Part 2 : Technique using rabbit plasma fibrinogen agar medium' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of Food Microbiology Sectional Committee and approval of the Food and Agriculture Division Council.

This standard was originally published in 1970 and first revised in 1976 when it was split into seven parts : Part 2 covering the methods for detection of both *staphylococci* and faecal *streptococci*. On a review by the technical committee responsible for formulating standards in this area, it was decided to revise this standard to separate the provisions and to align with the ISO Standards on the subject. Accordingly, Section 1 of Part 8 of IS 5887 covers enumeration of coagulase-positive *staphylococci* using Baird —Parker agar medium technique which is identical with ISO 6888-1 : 1999 and Section 2 of Part 8 of IS 5887 covers enumeration of coagulase-positive *staphylococci* using rabbit plasma fibrinogen agar medium technique which is identical with ISO 6888-2 : 1999. The provisions of faecal *streptococci* are retained as Part 2 of IS 5887.

In this adopted standard, certain terminology and conventions are not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'; and

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, the following International Standards are referred to. Read in their respective places the following:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 6887-1 : 1999 Microbiology of food and animal feeding stuffs — Rules for the preparation of the test sample, of initial suspension and of decimal dilutions for microbiological examination — Part 1 : General rules for the preparation of initial suspension and of decimal dilutions	IS 10232 : 1982 Guidelines for the preparation of dilutions for microbiological examination for food	Equivalent
ISO 6888-1 : 1999 Microbiology of food and animal feeding stuffs — Horizontal method for enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) — Part 1 : Technique using Baird-Parker agar medium	IS 5887 (Part 8/Sec 1) : 2002 Methods for detection of bacteria responsible for food poisoning : Part 8 Horizontal method for enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) Section 1 Technique using Baird-Parker agar medium	Identical

The technical committee responsible for the preparation of this standard has reviewed the provisions of ISO 7218 : 1996 'Microbiology of food and animal feeding stuffs — General rules for microbiological examinations' and has decided that it is acceptable for use in conjunction with this standard.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHODS FOR DETECTION OF BACTERIA RESPONSIBLE FOR FOOD POISONING

PART 8 HORIZONTAL METHOD FOR ENUMERATION OF COAGULASE-POSITIVE *STAPHYLOCOCCI* (*STAPHYLOCOCCUS AUREUS* AND OTHER SPECIES)

Section 2 Technique Using Rabbit Plasma Fibrinogen Agar Medium

1 Scope

This part of ISO 6888 describes a horizontal method for the enumeration of coagulase-positive staphylococci in products intended for human consumption or feeding of animals by counting of colonies obtained on a solid medium (rabbit plasma fibrinogen medium) after aerobic incubation at 35 °C or 37 °C (see reference [2]).

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 6888. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 6888 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Rules for the preparation of the test samples, of initial suspension and of decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and of decimal dilutions.*

ISO 6888-1:1999, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium.*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

3 Terms and definitions

For the purposes of this part of ISO 6888, the following terms and definitions apply.

3.1
coagulase-positive staphylococci
bacteria which form typical colonies in a rabbit plasma fibrinogen selective agar medium when the test is carried out according to the method specified in this part of ISO 6888

3.2
enumeration of the coagulase-positive staphylococci
determination of the number of coagulase-positive staphylococci found per millilitre or per gram of sample when the test is carried out according to the method specified in this part of ISO 6888

4 Principle

4.1 Preparation of duplicate poured plates of the rabbit plasma fibrinogen agar medium, with a specified quantity of the test sample if the product is liquid or with a specified quantity of the initial suspension in the case of other products.

Inoculation, under the same conditions, using decimal dilutions of the test sample or of the initial suspension, with two plates per dilution.

4.2 Incubation of the plates at 35 °C or 37 °C [1] for 18 h to 24 h, and a further 24 h if necessary.

4.3 From the number of typical colonies per Petri dish, calculation of the number of coagulase-positive staphylococci per millilitre or per gram of test sample.

5 Diluent and culture media

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Diluent

See ISO 6887-1 and the specific standard dealing with the product to be examined.

5.3 Rabbit plasma fibrinogen agar medium (see references [3] and [4]).

NOTE Commercially available media, in accordance with this part of ISO 6888, can be used. Nevertheless, considering the experienced variability of manufactured lots of the supplement, it is recommended that each batch of bovine fibrinogen/rabbit plasma solution be tested before use, by running positive and negative controls.

5.3.1 Base medium

Prepare the base medium as stated in ISO 6888-1:1999, 5.3.1, with the exception of the distribution of the base medium, in quantities of 90 ml per flask or bottle.

5.3.2 Solutions

5.3.2.1 Potassium tellurite solution

Prepare the potassium tellurite solution as indicated in ISO 6888-1:1999, 5.3.2.1.

5.3.2.2 Bovine fibrinogen solution

5.3.2.2.1 Composition

Bovine fibrinogen	5 g to 7 g [1]
Sterile water	100 ml

1) Depending on the purity of the bovine fibrinogen.

5.3.2.2.2 Preparation

Under aseptic conditions, dissolve the bovine fibrinogen in the water just prior to use.

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1) The temperature is agreed between the interested parties and is indicated in the test report.

5.3.2.3 Rabbit plasma and trypsin inhibitor solution

5.3.2.3.1 Composition

Rabbit plasma with EDTA for coagulase (EDTA coagulase plasma)	30 ml
Trypsin inhibitor	30 mg

5.3.2.3.2 Preparation

Operating under aseptic conditions, dissolve the components in the water, just prior to use.

5.3.3 Complete medium

5.3.3.1 Composition

Base medium (5.3.1)	90 ml
Potassium tellurite solution (5.3.2.1)	0,25 ml
Bovine fibrinogen solution (5.3.2.2)	7,5 ml
Rabbit plasma and trypsin inhibitor solution (5.3.2.3)	2,5 ml

5.3.3.2 Preparation

Melt the base medium, then let it cool down to 48 °C ± 1 °C in a water bath (6.3).

Under aseptic conditions, add the three solutions previously warmed to 48 °C ± 1 °C in a water bath. Mix thoroughly after each addition by rotation to minimize foaming.

Use the complete medium **immediately after its preparation**, in order to avoid any precipitation of the plasma.

WARNING If a commercially available solution of bovine fibrinogen/rabbit plasma is used, follow with great care the manufacturer's instructions for the preparation of this solution and of the complete medium (in particular the temperature of the base medium). Otherwise, the medium can completely lose its activity.

5.4 Preparation of agar plates

See ISO 6888-1:1999, 5.3.4.

6 Apparatus and glassware

NOTE Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) and **wet sterilization (autoclave)**

See ISO 7218.

6.2 Incubator, for maintaining the inoculated media, plates and tubes within the temperature range 35 °C ± 1 °C or 37 °C ± 1 °C.

6.3 Water bath, or similiar apparatus, capable of being maintained at 48 °C ± 1 °C.

6.4 Petri dishes, sterile, made of glass or plastic.

6.5 Total-delivery graduated pipettes, of nominal capacities 1 ml, 2 ml and 10 ml, graduated in 0,1 ml, 0,1 ml and 0,5 ml divisions, respectively.

7 Sampling

Sampling is not part of the method specified in this part of ISO 6888. If there is no specific International Standard dealing with sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned. If there is no specific International Standard available, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 Test portion, initial suspension and dilution

See ISO 6887-1 and the specific standard appropriate to the product concerned.

9.2 Inoculation and incubation

9.2.1 Take two sterile Petri dishes (6.4). Transfer, by means of a sterile pipette (6.5), 1 ml of the test sample if the product is liquid, or 1 ml of the initial suspension in the case of other products, to each of the dishes. Take two other sterile Petri dishes and transfer 1 ml of the first decimal dilution to each of the dishes.

Repeat these operations with successive dilutions using a new sterile pipette for each decimal dilution.

9.2.2 Into each Petri dish (9.2.1), immediately pour freshly prepared complete medium (5.3.3) (do not keep this in a liquid form) to a depth of approximately 3 mm.

Carefully mix the inoculum with the culture medium and leave to solidify by placing the Petri dishes on a cool horizontal surface.

9.2.3 After complete solidification, invert the thus prepared dishes and place them in the incubator (6.2) set at 35 °C or 37 °C [2)] for 18 h to 24 h. If necessary, re-incubate for 18 h to 24 h.

9.3 Counting of colonies

After a sufficient incubation period (see 9.2.3), the staphylococci form black or grey or even white, small colonies surrounded by a halo of precipitation, indicating coagulase activity. *Proteus* colonies may show, at the beginning of incubation, an appearance similar to coagulase-positive staphylococci colonies. However, after 24 h or 48 h of incubation, they may have the appearance of a spreading culture, more or less brownish, which allows them to be distinguished from staphylococci.

Count the typical colonies in each dish.

NOTE As the rabbit plasma fibrinogen agar is based on a coagulase reaction, it is not necessary to confirm this activity.

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2) The temperature is agreed between the interested parties and is indicated in the test report.

10 Expression of results

10.1 General case

Select those dishes containing at the maximum 300 colonies, with 100 typical colonies at two successive dilutions. One dish shall contain at least 15 typical colonies.

Calculate the number N of coagulase-positive staphylococci present per millilitre or per gram of product as a weighted mean from two successive dilutions using the following equation:

$$N = \frac{\sum C}{V(n_1 + 0{,}1\, n_2)d}$$

where

$\sum C$ is the sum of the characteristic staphylococcal colonies on all the dishes selected;

V is the volume of inoculum on each dish, in millilitres;

n_1 is the number of dishes selected at the first dilution;

n_2 is the number of dishes selected at the second dilution;

d is the dilution rate corresponding to the first dilution selected (the initial suspension is a dilution).

Round off the calculated results to two significant figures (see ISO 7218).

Take as the result the number of coagulase-positive staphylococci per millilitre (liquid products) or per gram (other products), expressed as a number between 1,0 and 9,9 inclusive, multiplied by 10^x where x is the appropriate power of 10.

EXAMPLE

A count of a product after inoculation with 0,1 ml of product gave the following results:

— for the first dilution selected (10^{-2}): 66 typical colonies and 54 typical colonies;

— for the second dilution selected (10^{-3}): 4 typical colonies and 7 typical colonies.

$$N = \frac{66 + 54 + 4 + 7}{2{,}2 \times 10^{-2}} = 5\,955$$

The result, after rounding off, is $6{,}0 \times 10^3$.

10.2 Estimation of low numbers

10.2.1 If the two dishes, corresponding to the test sample (liquid products) or the initial suspension (other products) each contain less than 15 colonies, report the result as follows.

a) For liquid products, estimated number of coagulase-positive staphylococci per millilitre:

$$N_e = \frac{C}{2}$$

where C is the sum of the colonies of coagulase-positive staphylococci counted (9.3) on the two dishes selected;

b) For other products, estimated number of coagulase-positive staphylococci per gram:

$$N_e = \frac{C}{2 \times d}$$

where

C is the sum of the colonies of coagulase-positive staphylococci counted (9.3) on the two dishes selected;

d is the dilution rate of the initial suspension.

10.2.2 If the two dishes, corresponding to the test sample (liquid products) or the initial suspension (other products) do not contain any coagulase-positive staphylococcal colony, report the result as follows:

— less than 1 coagulase-positive staphylococci per millilitre (liquid products);

— less than $1/d$ coagulase-positive staphylococci per gram (other products), where d is the dilution rate of the initial suspension.

11 Precision

See ISO 7218.

12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used, with reference to this part of ISO 6888;

— the incubation temperature used;

— all operating details not specified in this part of ISO 6888, or regarded as optional, together with details of any incidents which may have influenced the test results;

— the results obtained.

Bibliography

[1] KLOOS W.E. Systematics and the natural history of staphylococci. In: Staphylococci, *J. Appl. Bacteriol. Symp. Suppl.*, **69**, 1990, pp. 25 s - 37 s; and *Bergey's Manual of Determinative Bacteriology*, 9th edn., 1994.

[2] IDF 145A:1997, *Milk and milk products — Enumeration of coagulase-positive staphylococci — Colony-count technique.*

[3] BECKERS H.L. et al., Evaluation of a pour-plate system with rabbit plasma-bovine fibrinogen agar for the enumeration of *Staphylococcus aureus* in food. *Can. J. Microbiol.*, **30,** 1984, pp. 470-474.

[4] SAWHNEY D, The toxicity of potassium tellurite to *Staphylococcus aureus* in rabbit plasma fibrinogen agar, *J. Applied Bacteriology*, **61**, 1986, pp. 149-155.

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